Role of Propolis in Alleviation of Detrimental Effects of Salt Stress on Some Physiological and Cytogenetical Parameters in Onion (*Allium cepa* L.)

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Abstract
In this article, role of propolis on cytogenetic and physiological parameters in *Allium cepa* L. seeds exposed to salinity were studied. On the other hand, salinity showed a significant inhibitory effect on the seed germination and seedling growth of *Allium cepa*. Moreover, salinity reduced significantly the mitotic index in root tip cells and increased the chromosomal abnormalities and micronucleus which is the simplest indicator, the most effective of cytological damage. Althought the detrimental effects of salinity on the seed germination, mitotic activity, seedling growth and chromosomal aberrations were alleviated flashilly in varying degrees by propolis application, it was ineffective in reducing of salt damage on the micronucleus frequency.

Key words: Propolis, chromosomal aberrations, mitotic index, salt stress, seed germination, seedling growth

INTRODUCTION

Salinity affects plant metabolism in many aspects, reduces yield and growth. Excessive salt in the soil solution can adversely affect plant growth either through osmotic inhibition of specific ionic effects or water uptake of roots (Yildirim et al., 2006).

The pharmacological and chemical properties of propolis have been the target of intensive works in the last 30 years and the paradigm of propolis chemistry has changed drastically since the end of the 20th century. Although propolis may contain some pollen, it is not pollen and should not be confused with 'royal jelly' or 'bee bread', which are completely different products of the hive. Propolis, also known as 'bee glue', is the generic name for the resinous material obtained from various plant sources by honey bees from cones of trees, plant sprouts and the bark. Hence propolis extracts from different geographical origin have a specific combination of chemicals that reflect the floral properties of the field. Propolis have various biological and functional properties such as immuno-stimulating, antibacterial, antiviral, antimicrobial, hepatoprotective, anti-oxidative, anti-cancerious, tumoricidal, antimutagenic, antifungal, antiulceric as well as anti-inflammatory. Especially anti-inflammatory and anti-oxidative features of propolis make it a promising candidate as an adjuvant to chemotherapy (Uğurlu, 2013). As a result of the literature studies, no published studies on role of propolis on seed germination, seedling growth, micronucleus frequency, mitotic activity and chromosomal aberrations exposed to both salinity and normal conditions have been encountered. Therefore, this work is designed to investigate the effects of propolis in reducing of the harmful effects of salinity stress on cytogenetical and physiological parameters of *A. cepa* L.

MATERIAL AND METHODS

In the laboratory bioassay, *Allium cepa* L. seeds, 0.175 M NaCl and 100 mg L^{-1} propolis were used. Concentrations of salt and propolis were deter-

mined in a preliminary investigation conducted. This research work has done in Süleyman Demirel University Faculty of Art and Science Biology Department Plant Physiology and Cytogenetic Laboratories. This study, which started in September 2019 was completed in fifteen months.

Germination experiments were carried out with *A. cepa* seeds that are genetically and physiologically homogeneous. Onion seeds were germinated at a constant temperature (20°C) in the dark in an incubator. In the assay, seeds were sterilized with 2.5% NaClO solution for ten minute and washed in ultra-distilled water for 24 h. 20 seeds per each treatment group were placed in plastic containers. For seven successive days, they were divided into four groups:

- Group I (control) was exposed to distilled water
- Group II was exposed to 0.175 M salt alone
- Group III was exposed to 100 mg L^{-1} dose of propolis
- Group IV was exposed to 100 mg L^{-1} dose of propolis+0.175 M NaCl

It is assumed that the seeds in plastic containers placed in the incubator for germination should have length of 10 mm. Approximately 7 days after the beginning of the assay: final germination percentages were taken, radicle numbers were recorded and fresh weights were also determined. Entire experiments were repeated three times.

When the roots after several days reached 10.5 mm in length, they were excised, pretreated with saturated para-dichlorobenzene for four hrs, fixed in ethanol/acetic acid (3:1) solution for 24 hrs at room temperature for cytogenetic analysis. These fixed roots were stored in 70 % C_2H_6O, were hydrolysed with 5 N HCl by cold-hydrolysis method for 45 minutes, were stained in Feulgen for 1-1.5 hrs at room temperature, smashed in a drop of 45 % acetic acid, squashed then counted (micronucleus cells, mitotic aberrations and mitotic phases) in Olympus CX41 research microscope, they photographed (Olympus C-5060) at X500 magnification (Sharma and Gupta, 1982). A total of 1.000 cells were counted in each application group for the frequency of micronucleus (MN).

In addition to the evaluation of the induction of the chromosomal aberrations to in the study, mitotic inhibition (MI) approximately 2000 cells per each slides per sample were analyzed. The MI was calculated as the number of cells in mitosis divided by the total number of cells × 100%. The results were analyzed statistically (DMRT in SPSS). Micronucleus test is based on the criteria of Fenech et al. (2003). according to this:

i. MN should be 1/3 of the cell nucleus or smaller.

ii. MN should be round or oval.

iii. MN membrane should be clearly distinguishable from cell nucleus.

RESULTS AND DISCUSSION

The fresh weight and radicle number of group III seeds germinated in propolis medium decreased as compared with ones of the group I (control) seeds while their radicle length and germination percentage statistically were the same as ones of the group I seeds (Tab. 1). Conversely, there are certain studies about the effects of propolis on seedling growth and seed germination in normal conditions. But, it could not reach a consensus in these studies. Thus, propolis has been reported to inhibit (Derevici et al., 1964; Gonnet, 1968; Ghisalberti, 1979; Abdou and Omar, 1988; Zimonjic, 1989; Sorkun et al., 1997) and delayed (Sorkun and Bozcuk, 1994) the seedling growth, seed germination and mitosis. Some researchers confirmed various propolis applications like argentine propolis, brazilian green propolis against DNA damages in *A. cepa* have a protective effect. Propolis, is seen as a mutagenic agent due to the increase in micronucleus ratio, use in high doses due to micronucleus-enhancing effects can have harmful consequences (Tavales et al., 2006; Pereira et al., 2008). These differences of observations may have resulted from concentrations used, plant species and differences in treatment times.

NaCl showed an inhibitory effects on all growth parameters were re-emphasized with this study. For instance, control seeds germinated in distilled water after 7 days showed germination 100%, whereas this value was 23 % in group II seeds germinated at 0.175 M salinity. That is to say, NaCl prevented 77 % *A.* seed germination. Salinity stress can be preventive in many ways. Seed germination can be prevented by causing to change in water situation of the seed, thus prevent water intake (Ali, 2000). Results of the present study displaying the diminish in water content of the seedlings and the fresh weight

in salted conditions can be expressed by the inability of roots to receive enough water due to high of osmotic pressure in medium. Salt inhibitive effect on the radicle number, fresh weight and radicle length may result from reducing protein synthesis, nucleic acid and cell division. Salt stress's inhibitive effect on the seed germination was mitigated markedly by propolis application. Propolis continued also its success on seedling growth parameters like the fresh weight and radicle number except the radicle length. While group II's the radicle number and fresh weight were determined to be 12.7 and 7.0 g, respectively, these values were determined as 25.0 and 10.5 g in propolis + NaCl applied group (Tab. 1). As a result of the literature studies, literature in connection with effects of propolis on seedling growth and seed germination exposed to saline conditions have not been encountered.

This propolis alleviates salt stress on seedling growth and seed germination can be noticed by decreasing the osmotic effect of salt. For example, at 0.175 M salt application, propolis medium markedly increased growth parameters of seedlings compared to the control indicates this probability (Tab. 1). It reduced also the preventive effect of salt on seedling growth and seed germination by stimulating mitotic activity of the embryo (Tab. 2).

Çavuşoğlu et al. (2020a; 2020b) state that if stress conditions are present in the environment, any plant growth regulator should be added as in the germination process and some growth regulators might cause particularly mitotic irregularities, cell distortions and chromosomal aberrations even without stress conditions. There is only one study relating to the effects of propolis on the chromosomal aberrations and mitotic activity (Roberto et al., 2006). For this reason, this study was examined the first time whether propolis affected these parameters at normal and salinity conditions. According to results acquired from this experiment, the frequency CAs and MN in *A. cepa* meristem cells (group III) seeds exposed to 100 mg L^{-1} dose of propolis application flashily ascended according to ones of the group I seeds (Tab. 2). As a result of the literature works,

Table 1. Effect of propolis on some growth parameters of *Allium cepa* L.

Groups	Growth parameters			
	Germination percentage (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0^{c}	63.5 ± 0.5^{c}	63.2 ± 0.6^{d}	14.2 ± 0.8^{d}
Group II	23 ± 2.8^{a}	10.3 ± 0.3^{a}	12.7 ± 0.5^{a}	7.0 ± 0.5^{a}
Group III	100 ± 0.0^{c}	63.6 ± 0.7^{c}	57.5 ± 1.0^{c}	12.7 ± 0.3^{c}
Group IV	78 ± 2.8^{b}	13.3 ± 0.5^{b}	25.0 ± 0.5^{b}	10.5 ± 0.5^{b}

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 100 mg L^{-1} dose of propolis and Group IV: 100 mg L^{-1} dose of propolis+0.175 M NaCl.

Table 2. Effect of propolis on some cytogenetic parameters of *Allium cepa* L.

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberration (%)
Group I	*11.6 ± 1.0^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Group II	1.2 ± 0.2^{a}	13.0 ± 1.0^{c}	17.0 ± 0.4^{c}
Group III	12.2 ± 0.4^{c}	6.6 ± 0.5^{b}	16.1 ± 0.7^{c}
Group IV	3.1 ± 0.4^{b}	22.0 ± 2.0^{d}	7.9 ± 1.0^{b}

* The difference between the values in each column and the same letters isn't significant at the 0.05 level (±SD). Group I (control): distilled water, Group II: 0.175 M NaCl alone, Group III: 100 mg L^{-1} dose of propolis and Group IV: 100 mg L^{-1} dose of propolis+0.175 M NaCl.

although there is only one study in humans showing that propolis increases micronucleus frequency compared to control group (Eroğlu et al., 2004), there was no study showing that propolis increased the micronucleus frequency compared to the control group in plants.

Mitotic index can be used as a biological marker of increse cell growth in measuring the percentage of cells in different mitotic stages. A significant portion of the genetic damage produced by most mutagenic agent constitutes an important part of the chromosomal abnormalities (Kaymak, 2005). Some researchers have reported that high salt concentration causes total chromosomal abnormalities and mitotic activity inhibition in root tip meristematic cells (Radic et al., 2005). It is clear from the results of the present study that salt is cytotoxic on the meristematic cells of the tested plant. The data indicated that salinity according to the control decreased 89 % mitotic index, showed higher number of the chromosomal abnormalities and micronuclei. For example, the frequency of micronucleus and chromosomal abnormality in the root tip meristems of the seeds germinated in distilled water were 0.0 % and 0.0 % respectively while it were 13.0 % and 17.0 % at 0.175 M salt. In addition to, simultaneous propolis+NaCl application can be successful in alleviating of negative effect of salinity on the chromosomal aberration and mitotic activity but was observed increased the micronucleus frequency. Statistically, this performance was not be successful in decreasing of detrimental effects of salt on frequency of micronucleus. The cause of these high abnormalities and micronuclei may be due to salinity as mentioned above (Tab. 2). These results showed that propolis had a role in repairing against salt injuries during *Allium* mitosis.

Figure 1 showed normal mitotic phases observed during microscopic examination of *A. cepa* root tip meristem cells. Changes in chromosome structure due to exchange or a break in chromosome material are chromosomal abnormalities. Examples of alterations in the *A. cepa* demonstrated in Fig. 2. The most observed abnormalities are micronucleus, loss and vagrant chromosomes. Some rare abnormalities like cells with damaged nucleus, buds, nuclear peak, spindle disturbance, metaphase with breaks, exposure of chromosome scaffold, go into division of metaphase plate, alignment anaphase, later segregation, diagonal at anaphase, telophase chromosome protruded out, pole deviation in telophase could also be observed. In addition, salt formed cells with damaged nucleus (Fig. 2b) indicating its action on the mitotic spindle. The formation of budding nucleus (Fig. 2c) and micronuclei (Fig. 2a,m) suggest that constraints in the salt may be closely related to the loss of genetic material. Vagrants and laggards (Fig. 2 n,o) are a result of a partial despiralization of chromosomes.

Like mitotic irregularities diagonal at anaphase (Fig. 2l), alignment anaphase (Fig. 2ı) and bridges can basically result spindle dysfunction and constitutes an important part of chromosomal deviations. Micronucleus formations, the most effective and simplest endpoint for analyzing mutagenic effects, probably result of vagrant chromosomes and fragments. Later segregation and chromosome loss (Fig. 2j,k) are typically alterations associated to the malfunction of the mitotic spindle (Leme and Marin-Morales, 2009). Shimizu et al. (2000) stated that a consequence of cellular activities that promote the elimination of amplified genetic material can be nuclear buds (Fig. 2c). Spindle disturbance (Fig. 2e) can lead to micronucleus in the next stage

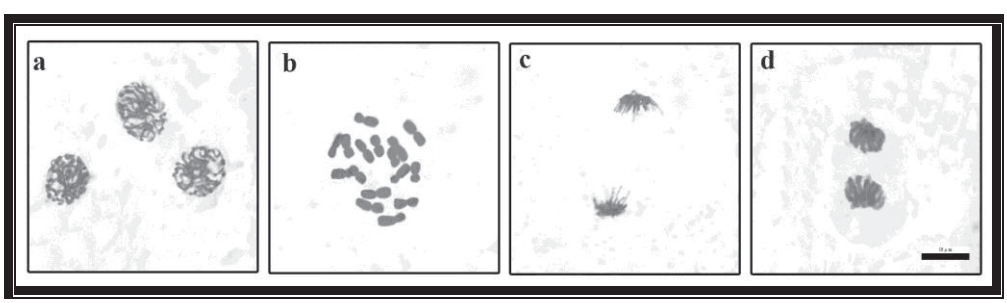

Figure 1. Normal mitosis phases in meristems of *A. cepa* root root tip cells Scale bar = 10 μm. a) prophases b) 2n = 16 chromosomes metaphase c) anaphase and d) telophase

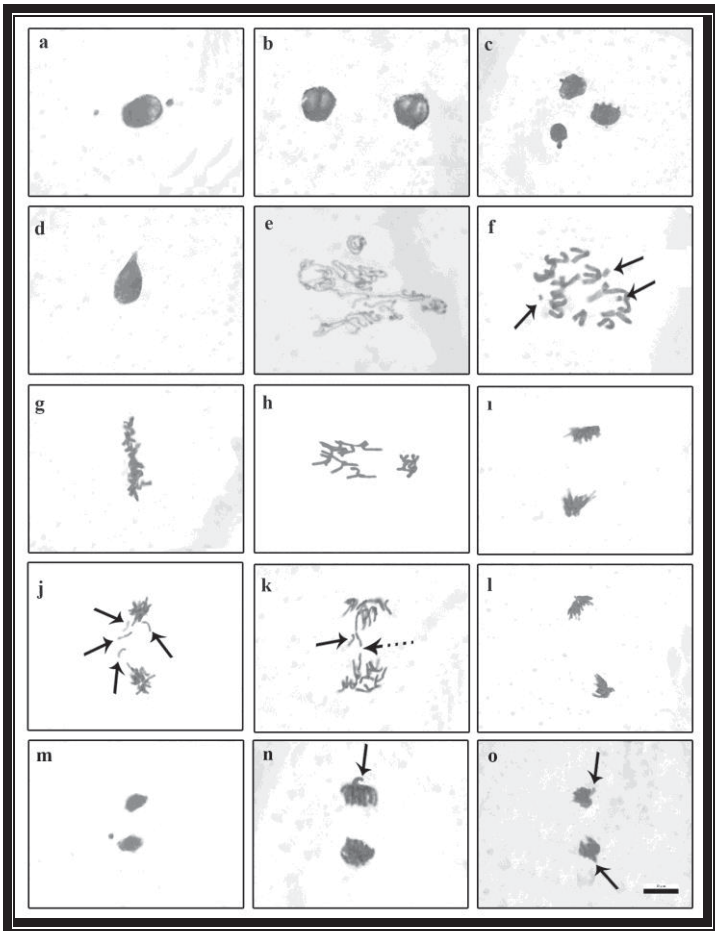

Figure 2. Chromosomal alterations examined in mitotic phases of *A. cepa* root tip cells. Scale bar = 10 µm. (a) micronucleus, **(**b) cells with damaged nucleus, (c) buds, (d) nuclear peak, (e) spindle disturbance, (f) metaphase with breaks, (g) exposure of chromosome scaffold, (h) go into division of metaphase plate, (ı) alignment anaphase, (j) indicate chromosome losses, (k**)** laggard = patterned and loss =arrow at anaphase, (l) diagonal at anaphase, (m) MN with telophase, (n) telophase chromosome protruded out, (o) vagrant chromosomes with pole deviation in telophase

of cell division. This is usually due to the irregular separation of chromosomes in anaphase and some chromosomes are allowed to reach the poles before the other. Chromosome breaks (Fig. 2f) known as clastogenic abnormalities and their action on chromosomes is generally regarded to involve an action on DNA. Thus the formation of chromosome breaks may be independent of the effect on the amount of DNA (Chauhan and Sundararaman, 1990).

CONCLUSIONS

Literature data on role of propolis application on cytogenetic and physiological parameters examined in salinity conditions have not been encountered as a result of the literature works. It is for this reason that the results of this study, especially in salinity conditions, are importance. As a conclusion, this study shows that propolis can significantly increase activations like seedling growth and seed germination under salt or alone conditions. However, mechanisms in which salt inhibits growth are controversial and complex. They can also vary by cultivar and species. So far, an universal mechanism has not been established. In spite of characterized salinity causes, the understanding of the salt prevent mechanisms plant growth remains weak. It is for this reason with further work is needed to learn more knowledge about the effect of propolis on the cell cycle, cell division and molecular metabolism of germination. In summary, this study to design

salt tolerance hypotheses in plants can serve to provide new conceptual tools.

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